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SYNTHESIS, PROPERTIES, AND DETOXIFYING ACTIVITY OF ANALOGS AND DERIVATIVES OF α-TOCOPHEROL

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In spite of the progress achieved in the treatment of pathologies induced by chemicals, problems in the search for new detoxifying materials still remain as before with an urgency caused by the growing volume of derivatives of chemical substances and the increasing number of people in contact with it. It is known [2] that one of the most active antioxidants, α-tocopherol (vitamin E), exerts a protective effect against poisoning by some chemical compounds, carbon tetrachloride in particular.

The metabolism of tocopherol is accompanied initially by oxidation of the phenyl group with either preservation or cleavage of the chromane ring, which leads to the formation of free or substituted o- or p-quinones [17]. The lateral carbon chain (C_{16}) on the 2-position of the chromane nucleus also may undergo change in the course of further transformations, accompanying the formation of new active form [15]. The metabolism of the quinonoid compounds formed, including the transformation of carbonyl group and the unsubstituted position of the quinone ring with subsequent destruction or formation of new biologically active form, is practically unknown.

At the present time, specific material to define the connection between structure and activity in the modified tocopherol series has been accumulated [1]. However, these data are all insufficient for the prediction of biological activity of newly synthesized compounds. This is a result of both nonsystematic and inconsistent data available by undirected syntheses and product screening results, including possible metabolites of tocopherol and other parts of the molecule, and the wide spectrum of physiological action and specific and other properties of the formed compounds, which also impede the evaluation of structure – activity relationships. New data on the biological properties, in particular on the detoxidying activity of structural analogs and derivatives of tocopherol, were therefore decidedly of interest.

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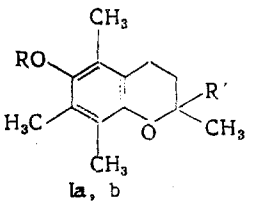
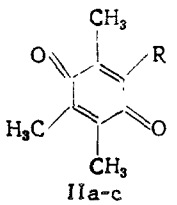
In the present work, we reduced the problem of the dependence of detoxifying properties to changing specific structural elements of the α-tocopherol molecule, specifically to the formation of new sterically-hindered phenol-containing compounds. Taking into account the significant antioxidant properties of α-tocopherol and the potential for these properties in its analogs and derivatives, their detoxifying activity was studied through the toxic manifestations of a substance giving the prooxidative effect. Studies carried out earlier [4] showed experimentally that significant pro-oxidative effects were observed with dinitroorthocresol (DNOC), widely used in the USSR state economy as a pesticidal preparation with a wide spectrum of activity.

The compounds used in this study were synthesized earlier: Ia [14], Ib [13], IIa [16], IIc and IVa [3], IIIa, b, and IVb [10], IIIc [7], IIId [8], Va [5], Vb and d [9], and Vc



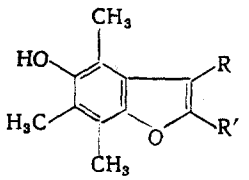
Ia:R = $COCH_3$, R′ = $CH_2CH_2CH{=}C(CH_3)_2$;
Ib:R = H, R′ = CH_3.

Ia, b



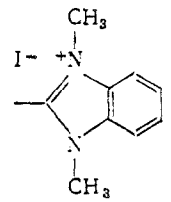
IIa:R = $CH_2CH_2C(CH_3)(OH)C_{16}H_{33}$;
IIb:R = $CH_2CH_2C(CH_3)(OH)CH_2CH_2CH{=}C(CH_3)_2$;
IIc:R = $CH(COCH_3)_2$.

IIa-c

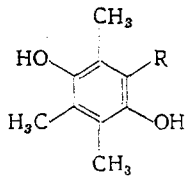


IIIa:R = H, R′ = CH_3;
IIIb:R = H, R′ = C_6H_5;
IIIc:R + R′ = $CH_2CHCHC(O)CH_2CH_2$

IIIa-d

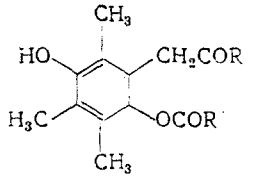


IIId:R = R′ = NH_2



IVa:R = $CH(COCH_3)_2$; IVb:R = CH_2COCH_3.

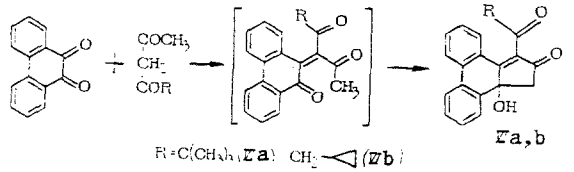
IVa, b



Va:R = R′ = CH_3; Vb:R = CH_3, R′ = C_6H_5;
Vc:R = R = C_6H_5; Vd:R = C_6H_5, R′ = CH_3.

Va-d

[6]. The phenanthrene derivatives VI also were studied. They were prepared by the interaction of phenanthrene 9,10-quinone with the corresponding β-diketone in the presence of triethylamine or piperidine.



VIa,b

The study of compounds VIa and b provides, to begin with, a clarification both of the biological spacial hindrance of the o-quinonoid structure, and the influence of methyl substitution on the phenolic portion of the molecule (compounds I-V) on the phenylene substituent.

The IR spectra of compounds VI shows the absorption bands of the OH-group at 3500 cm^{-1}, and three bands in the 1620-1710 cm^{-1} region related to the valence oscillations of the C=O groups. On the basis of these data, it can be proposed that the reaction does not stop at the stage of the formation of the tricyclic compounds (bracketed in the scheme), but proceeds further, accompanied by nucleophilic "attack" of the methyl group of the electron-deficient carbon atom of the free carbonyl group with subsequent cyclization to form the tetracyclic tautomers VIa and b. The PMR spectrum of compound VIa shows a singlet for the protons of the t-butyl group at 0.87 ppm (9H), a doublet of doublets for the protons of the CH_2-group at 3.38 ppm (1H) and 2.86 ppm (1H), a multiplet of the aromatic protons in the 7.2-8.12 ppm (8H), and also a singlet for the OH proton at 6.06 ppm.

EXPERIMENTAL (CHEMICAL)

IR spectra were recorded with an UR-20 (GDR) spectrophotometer in KBr pellets. The PMR spectra of compound VIa was obtained with a pulsed Fourier Bruker WP-200 spectrometer (FRG) at 200 MHz on a 0.2 M solution in $(CD_3)_2SO$ with internal HMDS as standard.

3,4-Dihydro-1-pivaloyl-2-oxo-4-hydroxycyclopental[l]phenanthrene (VIa). A mixture of 2.1 g (0.01 mole) of 9,10-phenanthrenequinone, 1.8 g (0.013 mole) of 5,5-dimethylhexan-2,4-dione, and 1.2 ml of triethylamine or piperidine in 10 ml of isopropyl alcohol was boiled for 15-20 min until complete solution of the quinone and formation of a dark red solution. The reaction mixture was kept overnight at room temperature, 1.5 ml of glacial acetic acid was added and the mixture was poured into 200 ml of water. The resulting product was filtered off and washed with water to give 2.4 g (74%); mp 185°C (from ethanol). Found, %: C 79.56; H 6.21. $C_{22}H_{20}O_3$. Calculated, %: C 79.52; H 6.02.

Compound VIb was obtained by an analogous procedure from 2.1 g (0.01 mole) of 9,10-phenanthrenequinone, 1.6 g (0.013 mole) of 4-cyclopropylbutan-2,4-dione and 1.2 ml of triethylamine to give 2.5 g (79%), mp 160°C (from toluene). Found, %: C 79.67; H 5.00. $C_{21}H_{16}O_3$. Calculated, %: C 79.75; H 5.06.

2,(3-Oxy-3,7-dimethyl-6-octenyl)-3,5,6-trimethylbenzoquinone-1,4 (IIc) was obtained from Ic (R=H) [14] by the method of [16]. IR spectrum (film): 3490 cm^{-1} (OH group), 1650 cm^{-1} (C=O group). Found, %. C 74.87; H 9.13. $C_{19}H_{28}O_3$. Calculated, %: C 75.00; H 9.21.

EXPERIMENTAL (BIOLOGICAL)

The detoxifying activity of the compounds was studied on rats of both sexes weighing 160-210 g. The model for acute poisoning was the pathological process developing in the animals under the conditions of a double (with a 1-day interval) peroral introduction of DNOC (sodium salt) in the form of a 1% aqueous solution at a dose of 35 mg/kg. The subject compounds were introduced intramuscularly in a prophylactic attempt twice 24 and 3 h from the introduction of the toxic agent into the rat at a dose of 30 mg/kg as a 1% solution in sunflower oil. The detoxification activity of the compounds was evaluated by the onset of intoxication, the time required for loss of the animals, and the period for clinical pattern of poisoning. The control in each series of experiments was a group of animals treated only with DNOC. The data obtained were treated statistically [12].

Acute toxicity of the compounds was studied on mice of both sexes weighing 17-21 g by a single intraperitoneal injection with the help of the express-method of determining the average effective dose and its errors [11].

RESULTS AND DISCUSSION

It was experimentally demonstrated that practically all of the studied compounds possessed detoxifying activity with respect to DNOC, definitely depending upon chemical structure (Table 1). Thus, reduction of the lateral chain in position 2 of the chromane ring with simultaneous introduction of a double bond in this chain (Ia) led to some increase in protective effect compared to α-tocopherol, manifested also in delayed death of the animal and a somewhat more favorable course of clinical intoxication by DNOC. Yet complete liquidation of the side chain (Ib), as with cleavage of the chromane ring with formation of a p-quinone structure (IIa-c), does not substantially influence this activity, although the latter is somewhat less for the quinone IIc than IIa and b.

There also was interest in a study of the influence of the furan system in analogous structures on detoxifying activity. It was established that introduction of the furan systems (IIIa-c) did not increase the protective effect by comparison with Ib. However, in

TABLE 1. Toxicity and Detoxifying Activity of α--Tocopherols, Their Analogs, and Derivatives

Compound	LD_{50}, mg/kg	Survival, %		Treatment Effect, %	p
		control	treated		
α-Tocopherol	>10000	50,0	87,5	+37,5	<0.05
Ia	>5000	25,0	75,0	+50,0	<0.05
Ib	890,0 (780,0—1020,0)	50,0	75,0	+25,0	>0.05
IIa	>5000,0	50,0	87,5	+37,5	<0.05
IIb	>5000,0	50,0	87,5	+37,5	<0.05
IIc	410,0 (350,0—470,0)	50,0	62,5	+12,5	>0.05
IIIa	1410,0 (1200,0—1700,0)	50,0	75,0	+25,0	>0.05
IIIb	1030,0 (880,0—1180,0)	25,0	62,5	+37,5	<0.05
IIIc	>2000,0	25,0	50,0	+25,0	>0.05
IIId	35,5 (31,0—40,0)	25,0	100,0	+75,0	<0.01
IVa	325,0 (280,0—380,0)	50,0	25,0	—25,0	>0.05
IVb	1290,0 (1100,0—1500,0)	50,0	75,0	+25,0	>0.05
Va	205,0 (180,0—240,0)	25,0	100,0	+75,0	<0.01
Vb	1370,0 (1000,0—1900,0)	25,0	100,0	+75,0	<0.05
Vc	1410,0 (1200,0—1700,0)	25,0	87,5	+62,5	<0.05
Vd	1120,0 (980,0—1270,0)	25,0	50,0	+25,0	>0.05
VIa	515,0 (450,0—590,0)	62,5	75,0	+12,5	>0.05
VIb	355,0 (310,0—400,0)	62,5	50,0	—12,5	>0.05

Note. The confidence limits for mice by intraperitoneal injection are given in brackets.

this series it should be noted that the use of benzofuran IIIb with an electron-acceptor substituent not only increases the number of DNOC-poisoned rats surviving compared to IIIa, but manifests a more favorable influence on the clinical course of the intoxification. Particular attention is merited by compound IIId, as the iodide with a benzimidazole residue in position 3 of the benzofuran nucleus. This compound showed a sharply increasing therapeutic activity, which apparently is explained by the ability of the 2-aminobenzofuran ring of this compound to easily interrupt the final formation of the p-quinonoid structure [8]. This process is analogous to the initial stage of the metabolism of α-tocopherol, involving the formation of α-tocopherol quinone (IIa) [17].

Analysis of the influence of the hydroquinone systems IV on the course and yield of the dinitro-orthocresolic poisoning of the aniamls indicates that if compound IIc with a quinonoic structure shows weak detoxification properties, then the corresponding hydroquinone IVa makes the detoxification worse. And, on the other hand, hydroquinone IVb possesses detoxification activity not inferior to 6-hydroxychromanol Ib, which apparently indicates an approximately equal stability to the oxidizing agent and thus the existence of derivative IVb in the hemiketal cyclic form [10].

No less interest and effectivness is apparent in the phenolic analogs of α-tocopherol (Va-d). The activity of compounds Va-c is the highest and does not depend upon the electron acceptor properties of the acyl substituent. For Vd however, in which is the phenacyl substituent, with a high steric hindrance and a high electron-acceptance compared to the acetonyl fragment of compounds Va and b, the effect is decreased.

It is known that the substitution of the methyl groups on the chromane nucleus of α-tocopherol by other radicals leads to a reduction of E-vitamin and antioxidant activity [1]. A study of the detoxification activity of the new derivatives (VI) of phenanthrene as representative of o-quinonoid structures not containing the structural fragments of α-tocopherol or its analogs showed that they did not exhibit noticeable detoxifying activity, which is evidently explainable by the structural features of these compounds.

In tests of the acute toxicity of these potential prophylactic agents against DNOC poisoning, it should be noted that only the iodide IIId, possessing the highest activity of those studied, is distinguished by significant toxicity. Other compounds, according to All-Union State Standard 12.1.007-76, may fall in the 3rd-class of danger level, i.e., moderately hazardous substances. The compounds Ia, IIa, and IIb are less hazardous.

Thus, this study shows that the transition to simpler analogs of α-tocopherol or to separate fragments of the molecule leads to the strengthening of the detoxification activity of such compounds with respect to DNOC. This is the basis for proposing that the bioantioxidant properties of the tocopherols are properties not only by their p- and o-quinonoid metabolites but also by simple structural analogs.

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